

Brazilian Journal of Development

Electrical conductivity of coffee seeds in function of the number of seeds and imbibing period

Condutividade elétrica de sementes de café em função do número de sementes e período de embebição

DOI:10.34117/bjdv5n12-191

Recebimento dos originais: 15/11/2019

Aceitação para publicação: 13/12/2019

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ABSTRACT

Coffee farming is one of the most expressive agricultural activities in Brazil. Coffee is a perennial crop with recalcitrant seeds, therefore, seed germination is irregular and lengthy. A fast evaluation of the quality coffee seeds is of an extreme importance. Thus, the objective of this work was to evaluate electrical conductivity of coffee seeds in function of the number of seeds and the imbibing period. To perform the experiments coffee seeds from four cultivars were used: IPR98, IPR100, IPR105 and IPR106. In the initial characterization the water content, the first and the last counting of the germination test and the accelerated aging of the seeds were determined. To determine seed vigor in a fast and precisely manner, the bulk

electrical conductivity test was used, using replicates with 25 and 50 seeds at a temperature of 25 °C for the evaluation periods of 2, 4, 6, 8, 12 and 24 hours. A completely random design was used for the experiments. The electrical conductivity test is efficient to evaluate coffee seed vigor. Cultivar IPR98 showed more deteriorated seeds. The test performed with 25 seeds showed differences between cultivars with 24 hours of imbibing period. Using 50 seeds it is possible to evaluate the physiological quality of the cultivars starting at 4 hours of imbibing period.

Keywords: Coffea arabica, seed quality, vigor.

RESUMO

A cafeicultura brasileira é uma das atividades agrícolas de maior expressão nacional. É uma cultura perene e suas sementes são recalcitrantes, por esse motivo sua germinação é lenta e desuniforme. A rápida avaliação na qualidade das sementes de café é de extrema importância. Assim, objetivou-se avaliar a condutividade elétrica de sementes de café em função do número de sementes e período de embebição. Para a realização dos experimentos foram utilizadas sementes de café provenientes de quatro cultivares IPR98, IPR100, IPR105 e IPR106. Na caracterização inicial determinou-se o teor de água, a primeira e a contagem final do teste de germinação e o envelhecimento acelerado das sementes. Para a determinação do vigor das sementes de maneira rápida e precisa foi utilizado o teste de condutividade elétrica em massa, utilizando repetições com 25 e 50 sementes na temperatura de 25 graus nos períodos de avaliação de 2, 4, 6, 8, 12 e 24 horas. O delineamento foi inteiramente casualizado. O teste de condutividade elétrica é eficiente para avaliação do vigor de sementes de café. A cultivar IPR98 possui sementes mais deterioradas. O teste conduzido com 25 sementes diferenciou as cultivares com 24 horas de embebição. Utilizando 50 sementes é possível avaliar a qualidade fisiológica das cultivares a partir de 4 horas de embebição.

Palavras-chave: Coffea arabica, qualidade de sementes, vigor.

1. INTRODUCTION

The germination test is the official and standard procedure to evaluate seed capacity to produce a normal plant under ideal conditions. However, the extended time required to obtain the results constitutes a disadvantage for such procedure (MATTIONI et al., 2015). This happens especially in coffee seeds (*Coffea arabica* L.) which show uneven and slow germination rate and generally have a low conservation potential for storage, increasing seed exposure to adverse conditions of the environment, which may affect its vigor and survival (CLEMENTE et al., 2015).

One of the main requirements to evaluate seed vigor is to obtain trustful and consistent results in a relative short period of time, accelerating decisions to be made. A fast evaluation of coffee seed quality is of an extreme importance to fulfill the demands from nurseries, coffee growers and laboratories. In order to verify the physiological quality of seeds which may be

used to produce seedlings it is essential to use quick and precise tests which may be easy to handle (REIS et al., 2010).

Electrical conductivity is one of the most studied fast tests for seeds and is related with the initial events of the deterioration sequence in seeds (DELOUCHE and BASKIN, 1973). Cell membrane disruption and the loss of its permeability control are the first processes characterizing deterioration. Such results are obtained by indirect evaluation of the cell membrane structuration degree through the determination of the quantity of leached ions in the imbibing solution, such as: sugars, amino acids, organic acids, proteins and phenolic substances, and inorganic ions such as: K^+ , Ca^{++} , Mg^{++} , Na^{++} (VIEIRA and KRZYZANOWSKI, 1999; MARCOS FILHO, 2015).

Electrical conductivity is a simple, cheap, objective and fast test and provides results in 24 hours (VIEIRA and KRZYZANOWSKI, 1999). This test may evaluate the quality of seeds while analyzing each seed in particular – Individual Conductivity Test - or analyze a sample and provide as a result a mean value for the solution conductivity where the leachates from the seeds constituting the sample are present – Bulk Conductivity Test.

Various factors may influence the results of the electrical conductivity test, among those the most important are genotype, membrane disruption, size and number of seed in the sample, imbibing temperature and period. These factors are subject of research in the pursue of more consistent results, specially the number of seeds in the sample and the imbibing period, which are considered extremely important factors (AVELINO et al., 2018).

Despite electrical conductivity test being considered one of the most rapid and promising tests for evaluation of seed quality, there is a lack of studies concerning its use in coffee seeds. Costa and Carvalho (2006) used the individual electrical conductivity test to evaluate coffee seeds, obtaining successful results in quality determination. However, there are no records of bulk conductivity test used to determine quality in coffee seeds. In this respect, the objective of the present work was to evaluate electrical conductivity of coffee seeds in function of the number of seeds and imbibing period.

2. MATERIAL AND METHODS

The experiment was performed in the Seed Laboratory from the Integrated Faculties from Ourinhos (FIO), Ourinhos-SP. Coffee seeds donated by the Agronomical Institute from Paraná (IAPAR), from four different cultivars: IPR98, IPR100, IPR105 and IPR106, were used to perform the experiments. Seeds were produced in the 2016 harvest.

Before starting the tests, parchments were manually extracted from seeds and discarded. The initial characterization of the cultivars was performed through the following tests:

Water content: executed in an oven at $105\pm 3^{\circ}\text{C}$, using two sub-samples of 25g of seeds each, according Brasil (2009).

Germination test: executed with four replicates of 50 seeds, planted in germitest blotting paper previously dampened with distilled water at a proportion of 2.5 mL.g^{-1} of paper. Seeds were kept in germination chambers at a constant temperature of 25°C . The evaluation consisted of two counts of normal plants, fifteen days (radicular protrusion) and thirty days after the tests began (BRASIL, 2009). Results were expressed as germination percentage (%).

Accelerated Aging: four replicates with 50 seeds were conditioned on web supports in germination boxes (gerbox), containing 40 mL of water under the web, and kept in aging chamber at $41^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and 100% relative humidity. After the aging period finished, the germination test was performed, under previously mentioned conditions, with scores determined after fifteen days and the results expressed as germination percentage (%).

The electrical conductivity test was performed with four replicates of 25 and 50 seeds each, weighted and conditioned in plastic cups with 200 mL capacity, containing 75 mL of deionized water. Then, the cups were kept in a B.O.D. chamber at 25°C , being evaluated in six imbibing periods 2, 4, 6, 8, 12 and 24 hours. Electrical conductivity was accessed with a Tecnal Tec-4MP digital conductivity meter. The equipment was calibrated with readings of up to $2000\text{ }\mu\text{S.cm}^{-1}$, variation of 2.02% and standard solution of $146.9\text{ }\mu\text{S.cm}^{-1}$ at 25°C . A completely random design was used, in a $4\times 2\times 6$ factorial, being four coffee cultivars (IPR98, IPR100, IPR105 and IPR106), two seed numbers (25 and 50) and six imbibing periods (2, 4, 6, 8, 12 and 24 hours). To statistically analyze data, the F test and variance analysis at 5% probability were used, when significant effects were detected means were compared by the Scott- Knott test at 5% probability, using the software SISVAR 5.0 (FERREIRA, 2011).

3. RESULTS AND DISCUSSION

According to the results showed in Table 1 significant differences were observed in physiological quality between coffee seeds. Data concerning the water content of seeds were similar among cultivars. This information is important, once uniformity of the initial water content of seeds contributes to obtain consistent results (LOEFFLER et al., 1988). Guedes et

al. (2011) observed that differences from 1 to 2% in water content between samples are not significant, that is, this percentage is considered within the range which do not influence the electrical conductivity test (HAMPTON, 1995).

Table 1. Initial characterization of physiological quality of coffee seeds.

Cultivar	Water Content (%)	Protrusion (%)	Germination (%)	Accelerated Aging (%)
IPR98	13.0	88 B	52 B	38 B
IPR100	13.2	97 A	80 A	36 B
IPR105	12.9	98 A	95 A	48 A
IPR106	12.8	95 A	71 A	35 B
CV (%)		3.37	8.16	5.23

*Means followed by the same letter within the row are not statistically different by the Scott-Knott test at probability.

The analyses of results from germination tests, radicular protrusion and accelerated aging showed that coffee seeds had differences regarding its physiological quality (Table 1). The germination test allowed determining that seeds from cultivar IPR98 had the lowest physiological quality when compared to the other cultivars. Analyzing radicular protrusion data, it was possible to verify cultivar IPR98 had the lowest quality. However, when the accelerated aging test was performed the best physiological quality was observed only in cultivar IPR105.

According to Araujo et al. (2011), seed lots with similar germination are fundamental in studies which focus on determining methods to evaluate seed vigor, once the objective is to separate lots of seeds with similar germination. If the germination potential of seeds shows accentuated differences, the germination test itself is able to detect differences in the physiological potential of the seeds (MARCOS FILHO and NOVENBRE, 2009).

Through the results of the analysis showed in Table 2, it is possible to observe significant differences between cultivars, number of seeds and imbibing periods, as well as for the interaction of the evaluated factors ($p < 0,05$). The results of the coffee seeds electrical conductivity comparing the number of seeds in function of the cultivars and imbibing periods are shown in Table 2.

Table 2. Electrical conductivity ($\mu\text{S cm}^{-1} \text{ g}^{-1}$) from coffee seeds in function of the number of seeds, cultivar and imbibing period (h).

Number of Seeds	Cultivars	Imbibing period (h)					
		2	4	6	8	12	24
25	98	26.6 Aa	45.0 Aa	55.2 Aa	75.4 Aa	96.4 Aa	176.6 Ab
	100	35.5 Aa	47.1 Aa	58.7 Aa	72.0 Aa	82.1 Aa	125.7 Aa
	105	35.9 Aa	63.9 Aa	83.5 Aa	100.1 Aa	127.1 Aa	205.4 Ab
	106	46.0 Aa	49.8 Aa	62.1 Aa	87.6 Aa	92.5 Aa	143.8 Aa
50	98	173.5 Aa	286.6 Bb	410.4 Bc	494.4 Bc	649.5 Bd	1138.4 Be
	100	77.6 Aa	104.7 Aa	127.3 Aa	132.4 Aa	183.4 Aa	314.2 Ab
	105	99.8 Aa	113.3 Aa	179.2 Aa	241.9 Ab	278.4 Ab	346.6 Ab
	106	74.7 Aa	110.0 Aa	135.4 Aa	167.0 Aa	207.4 Ab	300.2 Ab
CV (%)		39.31					

*Means followed by the same capital letter within the column and lowercase letter within the row are not statistically different by the Scott Knott test at 5% probability.

Regarding to the tests performed with 25 seeds, it was not possible to detect statistical differences between cultivars within each imbibing period, and therefore, it was not possible to differentiate the cultivars by its vigor level. Such similarities were verified through the electrical conductivity test while evaluating pepper seeds by Vidigal et al. (2008), while using 25 seeds was not efficient to separate lots in levels of physiological quality.

Analyzing the imbibing period allowed to verify statistical differences in cultivars IPR98 ($176,6 \mu\text{S cm}^{-1} \text{ g}^{-1}$) and IPR105 ($205,4 \mu\text{S cm}^{-1} \text{ g}^{-1}$) when using 24 hours of imbibing period and 25 seeds. For all other cultivars no differences in the quantities of leachates in function of the imbibing period were verified. Though, when comparing cultivars within the 24 hours period, one cannot support such cultivars showed higher deterioration than the others. These data differ from Oliveira and Novembre (2005), who while working with pepper seeds verified the higher vigor seed lot with only one hour of imbibing period.

When using 50 seeds to accomplish the test it was verified that leachates present in cultivar IPR98 statistically differed from the other cultivars with 4 hours of imbibing period onwards. The same cultivar did not show differences among the 6 and 8 hours of imbibition period, although there was an increment of leached electrolytes for 12 and 24 hours periods.

A substantial increase of leachates was observed with 24 hours of imbibing ($1138.4 \mu\text{S cm}^{-1} \text{ g}^{-1}$), differentiating cultivar IPR98 from the other cultivars and representing a higher deterioration of seeds. Deterioration process causes degenerative changes of the membrane system, reducing its integrity and/or selectivity, causing the loss of control over water and solute exchanges between cells and the exterior environment, thus determining the reduction

of seed viability (BINOTTI et al., 2008). The increase of leached electrolytes during the imbibing period was verified by different authors while using the electrical conductivity test to verify the physiological quality of seeds (VANZOLINI and NAKAGAWA, 2005; VIDIGAL et al., 2008).

Differences between the imbibing periods of 24, 8 and 12 hours were observed in cultivars IPR100, IPR105 and IPR106, respectively. Until six hours of imbibition period there were no changes in electrical conductivity of seeds from these cultivars. This enabled also to monitor the evolution of the deterioration process velocity of each seed individually and of each cultivar, once according Delouche (1975), the deterioration of the seed starts with the loss of the selective permeability from cell membranes and ends with the loss of germination potential.

Vidigal et al. (2008) found similarities in the ranking of seed lots for periods from one until seven hours of imbibing period, showing there was a tendency to preserve the electrical conductivity during these periods. Preserving the electrical conductivity was also observed from 8 until 24 hours of imbibing period in a study accomplished by Moura et al. (2017) with *Vigna unguiculata* seeds. The same tendency of preservation was verified in different coffee cultivars in the present study.

Data of electrical conductivity of coffee seeds in function of cultivars, number of seeds and imbibing period are shown in Table 3.

Table 3. Electrical conductivity ($\mu\text{S cm}^{-1} \text{ g}^{-1}$) from coffee seeds in function of cultivar, number of seeds and imbibing period (h).

Cultivar	Number of Seeds	Imbibing Period (h)					
		2	4	6	8	12	24
98	25	26.6 Aa	45.0 Aa	55.2 Aa	75.4 Aa	96.4 Aa	176.6 Ab
	50	173.5 Ba	286.6 Bb	410.4 Bc	494.4 Bc	649.5 Bd	1138.4 Be
100	25	35.5 Aa	47.1 Aa	58.7 Aa	72.0 Aa	82.1 Aa	125.7 Aa
	50	77.6 Aa	104.7 Aa	127.3 Aa	132.4 Aa	183.4 Ba	314.2 Bb
105	25	35.9 Aa	63.9 A	83.5 A	100.1 A	127.1 A	205.4 A
	50	99.8 A	113.3 Aa	179.2 Aa	241.9 Bb	278.4 Bb	346.6 Bb
106	25	46.0 Aa	49.8 Aa	62.1 Aa	87.6 Aa	92.5 Aa	143.8 Aa
	50	74.7 Aa	110.0 Aa	135.4 Aa	167.0 Aa	207.4 Bb	300.2 Bb
CV (%)		39.31					

*Means followed by the same capital letter within the column and lowercase letter within the row are not statistically different by the Scott Knott test at 5% probability.

Concerning to the number of seeds used in the conductivity test, differences between the cultivars evaluated were verified. In the case of cultivar IPR98 the use of 50 seeds increased the quantities of leachates starting with two hours of imbibing period. These results were similar to results described by Prete and Abrahão (1995) in coffee seeds, verifying the evaluation of the physiological differences was feasible starting at 3.5 hours while working with 50 seeds.

All other cultivars showed differences in electrical conductivity regarding the number of seeds. A clearest separation between cultivars in different vigor levels was possible also when using a higher quantity of seeds. This may be explained by the fact that a higher quantity of seeds produces a higher release of electrolytes (SILVA et al., 2014). When using a higher quantity of seeds, a better stratification in different vigor levels occurs in the seed lots (ARAÚJO et al., 2011).

It was possible to differentiate the number of used seeds in cultivar IPR100 with the electrical conductivity test only with 12 hours of imbibing period. The same phenomena occurred in cultivar IPR106, verifying differences in the electrical conductivity test when using 50 seeds with 12 hours of imbibing period. In cultivar IPR105 such difference was observed with 8 hours of imbibing period (Table 3). It is important to stress that a reduced imbibing period in coffees seeds to perform the electrical conductivity test was satisfactory, allowing to achieve faster results.

Xavier et al. (2017) established a period of 4 hours as sufficient to separate lots with 50 seeds of *Vigna unguiculata*. Vanzolini and Nakagawa (2005) evaluating electrical conductivity in peanut seeds, determined that statistical differences started to be observed with 3 hours of imbibing period. Using 50 coriander seeds, Torres et al. (2015), determined the evaluation of seed lot vigor to start with 2 hours of imbibing period. Thus, the number of seeds to be used in the electrical conductivity test will depend on the genotype and number of seeds to be used in the test.

4. CONCLUSIONS

Electrical conductivity test is efficient to evaluate coffee seed vigor. Cultivar IPR98 showed more deteriorated seeds in the test. The test performed with 25 seeds differentiated cultivars with 24 hours of imbibing period. It is possible to evaluate the physiological quality of cultivars for 4 hours of imbibing period when using 50 coffee seeds.

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